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Induction and Dorsoventral Patterning of the Telencephalon

Review

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The telencephalic vesicles are paired evaginations of the anterior forebrain that constitute the most complex and divergent structures in the vertebrate CNS. The principal components of the telencephalon are the pallium (primarily the cerebral cortex in mammals) and the subpallium (which constitutes most of the basal ganglia). Other telencephalic components, such as the septum and amygdala, have both pallial and subpallial origins. Telencephalic function is integrally dependent upon its connections with other neural structures including the thalamus, hypothalamus, olfactory epithelium, and brainstem. Together, these structures are essential for processing sensory information, integration of new sensory information with established memories (both experiential and instinctual), and then formulating and effecting behavioral responses. Thus, in humans, the telencephalon is the seat of consciousness, higher cognition, language, motor control, and emotions; damage to this structure leads to dementia, specific sensory and motor deficits, language and movement disorders, and changes in personality and emotional state. Although the telencephalon probably controls related functions in all vertebrates, neuroanatomists have yet to reach a consensus of opinion regarding the homologies between mature telencephalic nuclei and compartments in different classes of vertebrates. However, despite the highly variable morphologies of the adult telencephalon, it is now clear that the basic organization of telencephalic subdivisions is conserved during embryogenesis in all vertebrates.

Comparative studies of telencephalic development promise to yield important insights into brain patterning, function, and evolution. They will illuminate the conserved mechanisms that generate telencephalic properties common to all vertebrates, will establish the topological relationships between telencephalic subdivisions, and will determine homologies between telencephalic derivatives in different animals. Such studies will also elucidate the mechanisms by which telencephalic cytoarchitectures, connectivities, and functions have diverged during evolution. Understanding the cellular and genetic basis of telencephalic development promises to yield insights into the mechanisms underlying developmental disorders that in humans cause tragic illnesses such as mental retardation, autism, epilepsies, and childhood brain tumors. Ongoing research is also likely to establish the extent to which telencephalic development is regulated by intrinsic genetic mechanisms and to what degree it is malleable by extrinsic activity-dependent influences. Finally, understanding how the telencephalon is constructed may reveal new avenues for the development of therapeutic agents used to treat neurological and psychiatric disorders.

Until recently, studies of telencephalic development were primarily limited to morphological and neuroanatomical investigations. However, advances in genetic approaches, particularly in mice and zebrafish, are rapidly identifying genes that have crucial roles in forebrain development. These studies have started to unravel the genetic pathways that pattern the telencephalon and are also proving to be instrumental in reassessing the extent to which anatomical characteristics of the telencephalon are conserved or divergent in different classes of vertebrate.

Here we review recent studies that have revealed tissue interactions and signaling pathways involved in the early induction of the telencephalon and its subsequent subdivision into dorsal and ventral territories. We begin by describing genes that affect induction of the telencephalon, where new information has largely been gained through genetic screens in zebrafish and gain-of-function assays in amphibia. We finish by describing progress in our understanding of regional patterning of the telencephalon, where many of the advances have come from targeted mutagenesis experiments in mice. These studies have raised the intriguing possibility that early regional patterning may be linked, through the expression of particular transcription factors, to the specification of neuron-specific properties such as neurotransmitter phenotype.

Induction of the Telencephalon

The telencephalon derives from cells at the rostral margin of the neural plate (Rubenstein et al., 1998; Varga et al., 1999; Inoue et al., 2000; Whitlock and Westerfield, 2000). While minor differences are apparent in fate maps between species, in general, telencephalic precursors are situated rostral and lateral to prospective eye tissue, which itself is rostral to diencephalic territory. This places the telencephalic anlage at the margin of the anterior neural plate, where it is under the influence of signaling pathways that both regulate anterior-posterior (AP) and dorsal-ventral (DV) patterning of neural tissue.

The two-signal model of Nieuwkoop and colleagues proposes that induced neural tissue possesses anterior (forebrain) character and that a transforming (or posteriorizing) signal acts in a graded way to specify more posterior neural fates (Nieuwkoop et al., 1952; reviewed and updated in Foley et al., 2000). Candidate posteri-

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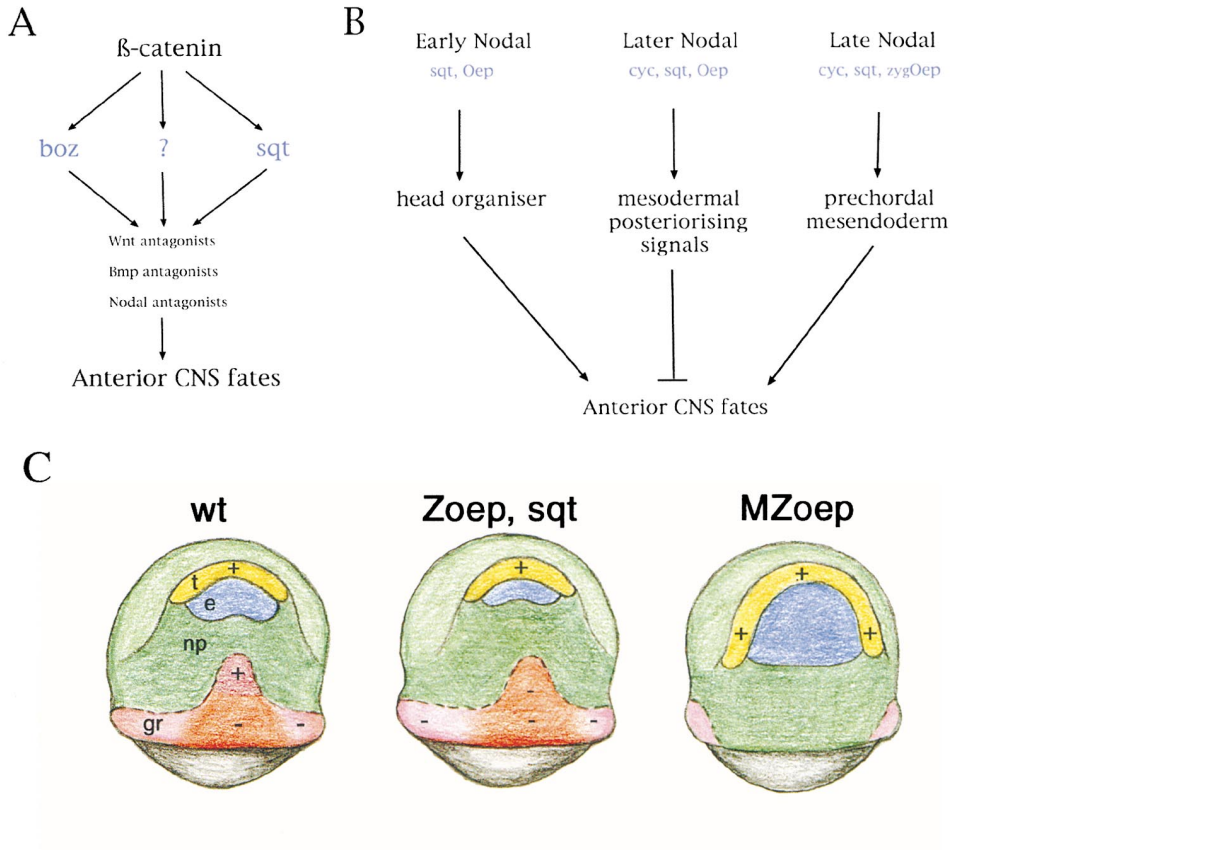

Figure 1. Early Specification of the Anterior Neural Plate in Zebrafish Embryos

(A) The homeoprotein Bozozok (Boz) and and Nodal ligand Squint (Sqt) act in parallel downstream of β-catenin to promote anterior neural fates. In embryos lacking both Boz and Sqt, loss of forebrain structures is much more severe than in single mutants alone (Shimizu et al., 2000; Sirotkin et al., 2000). These genes may promote anterior neural fates in part through the direct or indirect induction (arrows) of antagonists of Wnt, Bmp, and Nodal signals in cells on the dorsal side of the embryo (see text for references). They may also negatively regulate these pathways in other ways (e.g., Koos and Ho, 1999; Fekany-Lee et al., 2000, and not shown on diagram). The presence of patterned neural tissue in embryos that lack both Boz and Sqt suggests that there are probably other targets of β-catenin involved in regulating early neural patterning (see Shimizu et al., 2000). It has been proposed that Nodals, together with Activins may directly act as morphogens with forebrain fates specified at the lowest level of signaling activity (Thisse et al., 2000). This model seems at odds with the observations that reduction of Nodal activity through loss of Sqt function leads to loss rather than expansion of forebrain structures. However, this apparent contradiction might be reconcilable if a major role for Sqt is to induce inhibitors of Nodal activity in prospective anterior tissue (e.g., Meno et al., 1999) or if the loss of forebrain fates is due to reduced induction of neural tissue rather than a specific loss of anterior fates.

(B) Nodal signaling may act at different times and places to both promote and inhibit anterior neural fates. The earliest role of Nodal signaling may be to promote the development of a head organizer (Saude et al., 2000) that functions as a multimodal inhibitor of different signaling pathways in dorsal anterior regions (as shown in [A]; Sirotkin et al., 2000). Nodal signaling at this stage is probably primarily mediated by Sqt, but the variable reductions in anterior neural fates in *boz;cyc* embryos (Shimizu et al., 2000) suggests that the second Nodal ligand Cyc may also contribute to the induction of genes that promote forebrain development. In nonaxial regions, Nodal signaling has a more global role in mesoderm induction. Cell transplantation experiments have suggested that nascent mesoderm has posteriorizing activity (Woo and Fraser, 1997), and so loss of nascent mesoderm may result in expanded anterior neural fates (Koshida et al., 1998). Loss of Cyc activity in embryos that lack Sqt or lack both Boz and Sqt leads to expansion or restoration of anterior neural fates, respectively. This suggests that Cyc activity suppreses forebrain fates, perhaps indirectly through its role in mesoderm induction. Sqt activity in nonaxial cells may play a comparable role to Cyc in the supression of anterior fates, although this role has yet to be revealed genetically and may be masked by its role in axial regions in promoting anterior fates. All activity of Cyc and Sqt requires the function of the EGF-CFC protein Oep and embryos that lack both maternal and zygotic *oep* (MZoep) exhibit phenotypes equivalent to *sqt;cyc* embryos (Gritsman et al., 1999). At later stages, Nodal activity is required for maintenance of prechordal mesendoderm, which is reduced in *cyc*, *sqt*, and zygotic *oep* mutants. All these mutants show reduction in anterior CNS fates primarily restricted to ventral forebrain derivatives. However, some dorsal anterior forebrain structures are also variably reduced, particularly in zygotic *oep* embryos (Masai et al., 2000; K. A. Barth, K. Rohr, and S. W. W., unpublished data), suggesting that anterior axial tissues may continue to promote anterior forebrain fates during gastrulation.

(C) Schematics of dorsal views of gastrula stage zebrafish embryos illustrating possible roles of Nodal signaling in anterior neural plate patterning. The prospective telencephalon and anterior neural ridge are shown in yellow and the eye-forming region in blue. Germ ring tissue that involutes underneath the neural plate comprises prechordal mesendoderm (maroon), posterior axial mesendoderm (red), and nonaxial mesendoderm (pink). The relative positions of neural derivatives are simplified to reflect their approximate positions at the end of gastrulation (e.g., Varga et al., 1999). The signaling events illustrated may occur at various times before and after the stage illustrated. In the wild-type (wt) situation, prospective and definitive prechordal mesendoderm may produce signals (+), which promote anterior neural plate fates (eye and telencephalon). Prechordal mesendodermal signals may antagonize other signals (−) from more posterior germ ring derivatives that inhibit anterior neural fates. Signals from the ANR (+) may also promote forebrain fates. In zygotic *oep* (Zoep) and *sqt* embryos, prechordal

orizing signals include Fgfs, Wnts, retinoic acid, and Nodal family TGFβ proteins. While evidence exists for the involvement of all such signals (Lumsden and Krumlauf, 1996, and see below), it has remained uncertain how these diverse molecules each contribute to this process. However, recent genetic studies have shown that mutations affecting the function of the embryonic organizer directly or indirectly disturb AP patterning of the neural plate and can lead to increased or decreased specification of forebrain fates.

bozozok (*boz*) encodes a homeodomain protein required for organizer induction and function in zebrafish (Fekany et al., 1999; Koos and Ho, 1999). Embryos homozygous for mutations in the *boz* gene exhibit a phenotype in which anterior neural plate fates are variably reduced or lost. This appears to be due to the disruption of two different functions of the organizer—antagonism of Bmp signaling and antagonism of Wnt signaling (Fekany-Lee et al., 2000; Figure 1A). First, the neural plate of *boz* mutant embryos is reduced in size, at least in part reflecting the role of the organizer in the induction and maintenance of expression of proteins that antagonize the antineuralizing activity of Bmps. Second, the neural plate is posteriorized such that midbrain fates are specified near the front of the CNS. Forebrain fates can be rescued in *boz* embryos by expression of Wnt antagonists (Fekany-Lee et al., 2000; Hashimoto et al., 2000) suggesting that Boz normally negatively regulates the Wnt pathway and that Wnt signaling inhibits forebrain fates. *wnt8* expression is expanded in *boz* mutant embryos consistent with the possibility that enhanced Wnt8 activity may contribute to the loss of forebrain fates. These conclusions support studies in frogs that have shown that blocking Wnt activity promotes head development (Niehrs, 1999; Kazanskaya et al., 2000) and reveal a mechanism through which inhibition of Bmp and Wnt signaling is coordinated during forebrain specification.

Direct genetic evidence that the Wnt pathway can inhibit anterior neural plate fates has come from the recent cloning of the *headless* (*hdl*) mutation in zebrafish (Kim et al., 2000). The *hdl* gene encodes Tcf3, a transcriptional repressor of Wnt target genes, and so it is likely that mutations in *hdl* result in overactivation of Wnt signaling. *hdl* null mutant embryos appear to lack the telencephalon altogether and also have reduced diencephalic fates. Although this study provides genetic confirmation that suppression of Wnt signaling is a prerequisite for telencephalic development, it remains to be determined where and when Wnt signaling needs to be suppressed during embryonic development.

Experiments using frogs have suggested that blocking Nodal activity is also a requirement for induction of the head (Piccolo et al., 1999), although confusingly, Nodal activity is required for head development in mouse (e.g., Varlet et al., 1997). In fish, Nodal activity is needed for the development of midline neural tissue (Schier and Shen, 2000; Shen and Schier, 2000), and recent studies have revealed a more complex role for this pathway in the development of anterior neural fates, including the telencephalon and eye (Figures 1A–1C). Similar to Boz, the Nodal-related protein Squint (Sqt) is required for development of organizer tissue, and embryos lacking both Boz and Sqt, have considerably reduced CNS tissue in which the telencephalon is absent. This presumably reflects the additive effects of the mutations upon organizer function (Shimizu et al., 2000; Sirotkin et al., 2000; Figure 1A). Much more surprising is the finding that embryos lacking Boz, Sqt, and an additional Nodal-related protein, Cyclops (Cyc), have even smaller brains, but within these tiny brains, the telencephalon is present (Sirotkin et al., 2000). This suggests that residual Nodal signaling (mediated by Cyc) suppresses telencephalic development in *boz;sqt* mutants.

Analysis of zebrafish embryos lacking Nodal activity has confirmed that Nodal signaling suppresses telencephalic development. One-eyed pinhead (Oep) is an EGF-CFC protein essential for the reception of Nodal signals (Zhang et al., 1998; Gritsman et al., 1999). Zebrafish embryos lacking Oep activity are insensitive to Nodal ligands and exhibit a phenotype very similar to embryos lacking both Cyc and Sqt function (Feldman et al., 1998, 2000; Gritsman et al., 1999). Such embryos have an enlarged telencephalon (Gritsman et al., 1999) and eye (Masai et al., 2000), confirming that Nodal activity does repress anterior neural plate fates. Although it remains uncertain how Nodal signaling affects anterior neural fates, one striking feature of Nodal-deficient embryos is the complete lack of head and trunk mesendodermal derivatives. If these germ ring–derived tissues are a source of posteriorizing signals as is suspected from transplant experiments (Woo and Fraser, 1997; Koshida et al., 1998), then their absence could lead to an anteriorized neural plate (Figures 1B and 1C). More controversial is the possibility that Nodals (together with Activins) may directly impart AP identity to neural plate tissue in a concentration-dependent manner (Thisse et al., 2000). Misexpression of different concentrations of Antivin, a Lefty family protein that blocks the function of Nodal and Activin ligands (Bisgrove et al., 1999; Meno et al., 1999; Thisse and Thisse, 1999), leads to progressive and graded posterior to anterior truncation of CNS structures (Thisse et al., 2000). At very high levels of *antivin* expression, embryos consist of little more than forebrain and eye. One interpretation of these observations is that Nodal/Activin signaling establishes a gradient of positional information along the entire animal to vegetal axis (approximating the future AP axis) and that fore-

mesendoderm is reduced or absent, and, at least in *oep* embryos, this is due to a fate switch to chordamesoderm (Gritsman et al., 2000). This may result in an increase in posteriorizing signals (−) and a reduction in antagonists of such signals. Embryos with reduced Nodal signaling primarily show a loss of ventral forebrain derivatives although if combined with mutations in the *boz* homeobox gene, there is much more substantial loss of forebrain fates (Sirotkin et al., 2000; Shimizu et al., 2000). In *cyc;sqt* double mutants or maternal/zygotic Oep mutants (MZoep), all head and trunk mesendoderm is absent (Feldman et al., 1998, 2000; Gritsman et al., 1999), and so signals that inhibit anterior neural fates may be lost with the consequence that forebrain structures are expanded. Figure modified from Masai et al., 2000.
Abbreviations: e, prospective eye; gr, germ ring; np, neural plate; t, prospective telencephalon.

brain fates are specified at the lowest point on this gradient (Thisse et al., 2000).

Taken together, studies in fish and frogs support a model of early neural patterning in which induced neural tissue will develop anterior character unless exposed to posteriorizing signals. Indeed, perhaps in all vertebrates, the primary role for signals that promote forebrain development may be to antagonize or otherwise negatively regulate factors that would posteriorize the anterior neural plate. In mammals, a possible source of antagonists of posteriorizing activity is the anterior visceral endoderm (AVE). Anterior fates including the telencephalon are missing in embryos carrying mutations in genes required for development of the AVE (Knoetgen et al., 1999; Perea-Gomez et al., 2000). Although it is uncertain how the AVE (and related tissues in other species) regulates head development (see Foley et al., 2000), it may produce signals, including Cerberus, a multifunctional antagonist of Wnt, Bmp, and Nodal proteins (Piccolo et al., 1999), and Dickkopf, an antagonist of Wnt signaling (Glinka et al., 1998; Niehrs, 1999; Pearce et al., 1999), that are required for the induction and/or maintenance of the adjacent anterior neural plate. The physical separation of the AVE from the Node organizer raises the possibility that the AVE represents a head organizer distinct and independent from the Node. However, analysis of mouse embryos lacking the function of Noggin and Chordin (which are expressed in the Node but not in the AVE), indicates that AVE development is dependent upon the Node (Bachiller et al., 2000). In mutant embryos, AVE markers are initially induced but are not maintained, and anterior neural fates are subsequently lost. As both Noggin and Chordin are Bmp antagonists, it is possible that Bmp signaling may directly regulate AP neural patterning. However, there is increasing evidence that the primary role of the Bmp pathway is to regulate the DV patterning of the neural plate rather than its AP patterning.

In zebrafish, Bmp activity is required for early telencephalic development (Barth et al., 1999). In the absence of Bmp2b/Swirl, the neural plate is expanded at the expense of nonneural ectoderm (e.g., Mullins et al., 1995). However, markers of lateral neural plate identities (including the telencephalon) are reduced or absent, whereas more medial/ventral neural plate fates, such as the prospective retina are expanded. This result initially appears at odds with observations in frogs in which suppression of Bmp activity through expression of Bmp antagonists can induce markers of telencephalic identity in explant assays (e.g., Lamb et al., 1993; Sasai et al., 1995). However, Bmp activity may normally establish a gradient of positional information across the neural plate and might therefore either promote or inhibit fates at specific DV positions dependent upon the level of Bmp activity that is present (Nguyen et al., 1998, 2000; Barth et al., 1999).

Although the Bmp pathway has profound effects upon early DV patterning, at least in fish, it appears to have no major role in AP patterning of the neural plate (Nguyen et al., 1998, 2000; Barth et al., 1999). It remains to be determined if the Bmp pathway in mice is more directly involved in specifying anterior neural fates as is suggested by the Noggin/Chordin loss-of-function phenotype. However, this phenotype could also be interpreted as a failure to maintain neural tissue in anterior regions rather than a failure to specify anterior positional values. Indeed, if a neural inducing signal is reintroduced to fish embryos that appear to lack head structures, then anterior neural fates are restored suggesting that anterior positional identities may still be present in embryos that lack overt head structures (Koshida et al., 1998, and see Ober and Schulte-Merker, 1999).

The Anterior Neural Ridge

Ablation, transplantation, and explant studies in mice and fish have suggested that subsequent to neural induction, cells at the rostral margin of the neural plate (the anterior neural ridge) play an important role in the development of the telencephalon (Shimamura and Rubenstein, 1997; Houart et al., 1998). Telencephalic gene expression is reduced or absent when cells at the margin of the prospective neural plate are removed in gastrula stage fish embryos, and these same cells can induce telencephalic gene expression when transplanted to more caudal regions of the neural plate. Furthermore, mouse explants in which the anterior neural ridge is removed fail to express the telencephalic marker *bf1,* again supporting the notion that one or more signals from the margin of the neural plate promote telencephalic development.

There is evidence that, in vitro, Fgf8 function is necessary and sufficient to regulate *bf1* expression. For instance, Fgf8 can restore *bf1* expression to mouse explants denuded of the anterior neural ridge (Shimamura and Rubenstein, 1997), and inhibitors of Fgf function reduce *bf1* expression in neural plate explants (Ye et al., 1998). Indeed, *fgf8* is expressed in cells at the margin of the neural plate and later at the midline of the telencephalon. However, *fgf8* expression is probably initiated too late to be the primary inducer of the telencephalon, and indeed much of the telencephalon is still present in mice (Meyers et al., 1998; E. Storm, J. L. R. R., and G. Martin, unpublished data) and fish (Shanmugalingam et al., 2000) with compromised Fgf8 function. Furthermore, anterior neural plate cells taken from fish embryos lacking functional Fgf8 still possess inductive activity (Shanmugalingam et al., 2000). These results suggest that, although Fgf8 does contribute to the signaling activity of anterior neural plate cells, alone it is probably not responsible for induction of the telencephalon. However, as several *fgf* genes are expressed in the forebrain (e.g., Wang et al., 2000), it remains possible that Fgf signaling has a more comprehensive role in telencephalic induction than is apparent from the loss of a single ligand.

One role of the anterior neural ridge may be to regulate the subdivision of the anterior neural plate into telencephalic, optic, and diencephalic domains. The *masterblind* (*mbl*) mutation in zebrafish disrupts this regional subdivision. In *mbl* mutant embryos, the telencephalon and eyes are respecified to more posterior diencephalic fates (such as the pineal organ), which are consequently expanded to the front of the forebrain (Heisenberg et al., 1996; Masai et al., 1997). This phenotype suggests that specification of telencephalic identity may require the suppression of posterior diencephalic identity. Gain- and loss-of-function studies in various species have

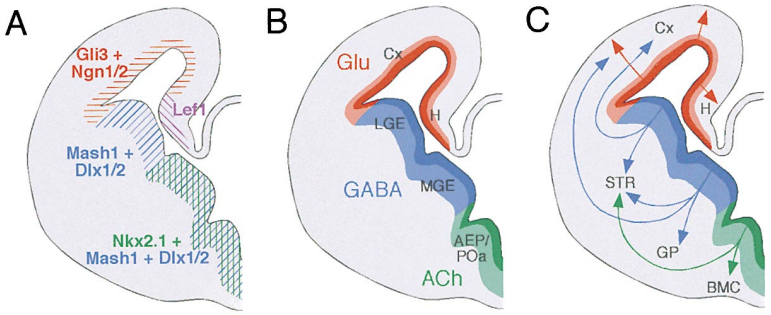

Figure 2. Production and Migration of Different Classes of Neurons at Different DV Positions within the Telencephalon

Schemas representing the right telencephalic hemispheres from E14 mouse embryos.

(A) Approximate expression patterns in the progenitor zones of selected transcription factors that are implicated in regulating telencephalic patterning and differentiation (see text).

(B) Major subdivisions of the telencephalic proliferative zone. The schema also shows the possible locations of precursor cells that produce neurons expressing the neurotransmitters, glutamate, GABA, and acetylcholine.

(C) Migration pathways of neurons that populate different telencephalic domains. The arrows indicate migration pathways from different progenitor domains. Straight arrows reflect radial migrations, whereas curved arrows correspond to tangential migrations. It is suggested that projection neurons generally follow radial migrations whereas interneurons (local circuit neurons) generally follow tangential migrations (Marin et al., 2001).

Abbreviations: Ach, acetylcholine; AEP, anterior entopeduncular area; BMC, basal magnocellular complex; Cx, cortex; GABA, gamma-aminobutyric acid; Glu, glutamate; GP, globus pallidus; H, hippocampus; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; POA, anterior preoptic area; STR, striatum.

identified a number of homeobox containing genes including *hesx* (Dattani et al., 1998; Ermakova et al., 1999), *rx* (Andreazzoli et al., 1999), *six3* (Kobayashi et al., 1998; Loosli et al., 1999), and *pax6* (Chow et al., 1999) that may have roles in mediating the early regional subdivision of the prospective forebrain into telencephalic, optic and diencephalic territories. Several other mutations that have yet to be cloned, such as *flat-top* and *oto*, also appear to be required for early telencephalic growth and patterning (Hentges et al., 1999; Zoltewicz et al., 1999).

Regional Patterning of the Developing Telencephalon

In all vertebrates, the developing telencephalon is subdivided into ventral (subpallial) and dorsal (pallial) territories. In mammals, the pallium primarily gives rise to the cerebral cortex, while the subpallium consists of the medial (MGE) and lateral (LGE) ganglionic eminences (Figure 2), which later form the striatal and pallidal components of the basal ganglia. While the morphology of adult pallial and subpallial structures varies enormously between species, very similar regional subdivisions are observed in the telencephalon of all classes of vertebrates during early development (Fernandez et al., 1998; Puelles et al., 2000). Indeed, it seems likely that the same signaling pathways regulate early regional patterning of the telencephalon from fish through to mammals.

The Hedgehog Pathway and Ventral Telencephalic Development

Shh is a member of the Hedgehog family of secreted signaling proteins implicated in a wide variety of developmental processes. Mice, fish, and humans that have defects in Shh signaling lack ventral telencephalic structures (Chiang et al., 1996; Muenke and Beachy, 2000), but it is uncertain how, when, or where Shh is required for telencephalic patterning. *shh* is expressed in the ventral telencephalon, but loss of this site of expression alone does not lead to the severe telencephalic defects observed in mouse *shh* mutants (Huh et al., 1999; Sussel et al., 1999). Therefore, either Shh secreted from some other region acts upon telencephalic cells or Shh is required for an earlier developmental event that secondarily affects telencephalic patterning.

Although genetic studies have yet to resolve the precise role of Shh in telencephalic patterning, gain-of-function and explant studies show that Shh can promote ventral telencephalic identity. For instance, ectopic expression of Shh in mice and fish induces ventral telencephalic markers such as *nkx2.1, gsh2*, and *dlx2* within dorsal telencephalic cells (Gaiano et al., 1999; Corbin et al., 2000), mimicking its inductive abilities in vitro (Ericson et al., 1995; Dale et al., 1997; Pera and Kessel, 1997; Shimamura and Rubenstein, 1997; Kohtz et al., 1998). In chick, explants of prospective telencephalic cells exposed to Shh during gastrulation stages initiate a program of differentiation that leads to expression of ventral telencephalic markers at much later stages (Gunhaga et al., 2000). In vivo, the most likely source of Shh at these very early stages is Hensen's node or the primitive streak, lending support to the idea that prospective telencephalic cells may be exposed to Hedgehog signals that originate from outside of the telencephalon itself.

Further elucidation of the role of Shh in telencephalic patterning will come from analysis of the functions of other components of the Hedgehog signaling pathway. Patched is a component of the Shh receptor complex and functions as a negative regulator of Shh signaling (Murone et al., 1999; Kalderon, 2000). In *patched1* mutant mice, expression of *nkx2.1* is expanded, consistent with the notion that localized activity of Shh in the ventral telencephalon normally induces this gene (Goodrich et al., 1997). The Gli family of transcriptional regulators are downstream effectors of Shh signaling (Ruiz-i-Altaba, 1999), and one might expect one or more Gli proteins to mediate ventral telencephalic patterning if Shh is active in this region. Indeed, ectopic Gli1 can promote *nkx2.1* expression (Ruiz-i-Altaba, 1998). However, loss-of-function studies indicate that, although Gli3 has a role in patterning the dorsal telencephalon (see below), as yet there is no clear evidence of a requirement for Gli3 or other Gli proteins in the establishment of ventral telencephalic territories (Matise et al., 1998; Park et al.,

2000). This raises the possibility that within the ventral telencephalon, Shh signaling may be mediated by transcription factors other than members of the Gli family.

Shh may also be involved in the subdivision of the ventral telencephalon into MGE and LGE. Several of the transcription factors downstream of Shh and key to the subdivision of the ventral telencephalon are homeodomain proteins including Nkx.2.1 (Kimura et al., 1996; Sussel et al., 1999), Gsh2 (Corbin et al., 2000; Torresson et al., 2000; Yun et al., 2001), and Pax6 (Stoykova et al., 2000). Within the ventral telencephalon, *nkx2.1* mutants have a ventral-to-dorsal transformation, and cells that should form MGE are transformed to an LGE identity. A related phenotype is observed in mice carrying mutations in *gsh2*, a homeobox gene expressed in both the MGE and LGE. In these mutants, there is ectopic expression of pallial markers in the dorsal LGE, indicating dorsalization of this region of the subpallium (Corbin et al., 2000; Torresson et al., 2000; Yun et al., 2001). Complementary to the *gsh2* and *nkx2.1* mutant phenotypes, mice lacking function of *pax6* express subpallial genes within the ventral pallium (Stoykova et al., 2000; Torresson et al., 2000; Yun et al., 2001) and MGE-specific genes in the LGE (Stoykova et al., 2000). Mice lacking both Pax6 and Gsh2 show milder phenotypes than single mutants (Torresson et al., 2000), confirming that reciprocal regulatory interactions between these genes mediate DV patterning on either side of the pallial/subpallial boundary. Together, these results suggest that homeobox gene activity mediates DV patterning of the telencephalon and establishes boundaries between regional subdivisions. This is highly reminiscent of the situation in more caudal regions of the CNS where graded Shh activity induces or represses the expression of various interacting homeobox genes that subsequently influence the production of neurons of different DV identities (Briscoe et al., 1999, 2000; Jessell, 2000).

The Fgf signaling pathway also appears to regulate patterning of ventral telencephalic structures. For instance, in fish, *nkx2.1* expression is reduced and ventral telencephalic midline tissue is disrupted in *ace* mutant embryos that lack Fgf8 function (Shanmugalingam et al., 2000). Indeed, more severe reduction of Fgf signaling activity leads to a greater reduction of ventral telencephalic markers confirming a role for Fgf signaling in promoting ventral telencephalic identity or growth (M. Shinya and H. Takeda, personal communication). However, other phenotypic defects in *ace* mutant embryos indicate that Fgf8 has widespread and complex roles in telencephalic patterning and differentiation. Telencephalic development in mice with mutations in the *fgf8* gene has yet to be studied in detail, although it is clear that, as in fish, there are ventral telencephalic and olfactory bulb deficits (Meyers et al., 1998; E. Storm, J. L. R. R., and G. Martin, unpublished data). As noted earlier, in vitro studies have suggested that one downstream target of Fgf signaling may be the winged helix transcription factor encoding gene *bf1* (Shimamura and Rubenstein, 1997). Studies in both mice and frogs suggest that Bf1 has roles both in regional specification and in the regulation of cell proliferation (Xuan et al., 1995; Dou et al., 1999; Huh et al., 1999; Hardcastle and Papalopulu, 2000).

Pathways Involved in Dorsal Telencephalic Development

Mutations in several transcription factors result in dorsal-to-ventral transformations within the telencephalon. Mouse *gli3* mutants lose expression of many dorsal telencephalic markers and structures (such as hippocampus) and in one genetic background, markers of the LGE spread into the cerebral cortex (Theil et al., 1999; Tole et al., 2000b). These changes may at least in part reflect an activity of Gli3 as a repressor of Shh target genes. Among the genes that exhibit reduced dorsal expression in *gli3* mutant mice is the homeobox gene *emx2*. In the dorsal telencephalon, *emx2* is expressed at higher levels in caudal than in rostral areas, and it has been proposed that graded Emx2 activity may confer regional identity within the cortex (Bishop et al., 2000; Mallamaci et al., 2000). In support of this, loss of Emx2 function leads to expansion of rostral/lateral cortical domains, while caudal/medial domains are reduced or lost (Pellegrini et al., 1996; Yoshida et al., 1997; Bishop et al., 2000; Mallamaci et al., 2000; Tole et al., 2000a). Conversely, cortical Pax6 expression is higher rostrally and ventrally than dorsally and caudally. In addition to the disruptions to ventral pallial development described above (Stoykova et al., 2000; Torresson et al., 2000; Yun et al., 2001), loss of Pax6 function also leads to expansion of caudal cortical domains at the expense of rostral domains (Bishop et al., 2000). Opposing activities of the homeodomain proteins Pax6 and Emx2 may therefore generate graded positional identity within the dorsal telencephalon and contribute more generally to the homeodomain protein "code" that appears to regulate overall regional subdivision of the telencephalon.

Although homeobox gene activity is crucial to telencephalic patterning, other families of transcription factor-encoding genes also profoundly influence development of this region of the brain. For instance, a striking dorsal-to-ventral fate change occurs in the telencephalon of mice with altered expression of bHLH family transcription factors (Fode et al., 2000). In both vertebrates and invertebrates, bHLH proteins regulate neurogenesis and influence neuronal identity (Jan and Jan, 1994). In the telencephalon, *mash1* is expressed ventrally, while two *neurogenin* (*ngn*) genes are expressed dorsally (Figure 2A). In *ngn2* or *ngn1;ngn2* mutants, there is ectopic induction of ventral molecular markers such as *mash1* in the cerebral cortex, indicating that Ngn activity promotes dorsal telencephalic development by suppressing ventrally expressed genes. Further experiments demonstrate that Mash1 is both required and is sufficient to confer ventral telencephalic identity to cortical neurons when ectopically expressed in the pallium. Although the loss of subsets of ventral neurons in *mash1* null mutant mice confirms a role for this protein in ventral telencephalic development (Casarosa et al., 1999), it remains to be resolved how Mash1 function relates to other genes involved in DV patterning. For instance, it may confer competence for ventral telencephalic cells to respond to patterning signals such as Shh, or, alternatively, it may function as a downstream effector of such signals (Fode et al., 2000).

In addition to their early functions during neural plate formation, the Bmp and Wnt pathways have later roles in patterning the pallium. Several Bmps are expressed

in and around the dorsal telencephalon, and studies using explant cultures and misexpression in vivo suggest involvement in the regulation of patterning, cell survival, and proliferation. Ectopic Bmp activity represses ventral telencephalic markers (such as *nkx2.1* and *dlx2*), while maintaining or increasing dorsal markers, and leads to decreased proliferation, increased apoptosis in the basal telencephalon, and holoprosencephaly (Furuta et al., 1997; Golden et al., 1999; Y. Ohkubo and J. L. R. R., unpublished data). Loss-of-function studies have yet to demonstrate a clear role for Bmps in DV patterning of the telencephalon but dorsal development is clearly disrupted in mice lacking both Bmp5 and Bmp7 (Solloway and Robertson, 1999). These mutants have delayed closure of the rostral neural tube, hypoplasia of the telencephalic vesicles, and reduced apoptosis in the telencephalic roof.

Like Bmps, several members of the Wnt family of secreted signaling proteins are likely to be involved in dorsal telencephalic development. Loss-of-function mutations in *wnt3a* and in a downstream effector of Wnt activity, *lef1*, lead to loss/reduction of the hippocampus (Galceran et al., 2000; Lee et al., 2000). Indeed, an allele of *lef1* that is predicted to widely interfere with the function of other transcriptional mediators of Wnt signaling also eliminates the hippocampus. Both *wnt3a* and *lef1* mutants have a modest reduction in cell proliferation in the hippocampal anlage, suggesting that Wnt signaling is required for expansion of this structure. Mice lacking Lhx5 also lack a hippocampus, but it has yet to be determined whether this Lim homeobox gene is regulated by Bmp and/or Wnt signals (Zhao et al., 1999).

DV Regionalization of the Telencephalon Is Linked to the Generation and Migration of Neurons Expressing Different Types of Neurotransmitters

While distinct patterning mechanisms regulate the specification of cerebral cortex and basal ganglia, recent studies have revealed an unexpected interdependence between these domains. This has been most strikingly demonstrated through analysis of the various tangential migrations of neurons between different telencephalic structures (Anderson et al., 1997a, 1997b; DeCarlos et al., 1996; Tamamaki et al., 1997; reviewed by Parnavelas, 2000; Figure 2C). The basal ganglia produce both projection neurons that migrate radially to their final locations (within the basal ganglia) and interneurons, many of which follow one of at least two tangential migrations through the basal ganglia to dorsal cortical structures (Anderson et al., 1997a, 1997b, 1999). Indeed, a lateral migration of cells from the MGE is the source of perhaps the majority of the GABAergic interneurons of the LGE/striatum and pallium/cerebral cortex (Lavdas et al., 1999; Sussel et al., 1999; Wichterle et al., 1999; Marin et al., 2000; Pleasure et al., 2000 [this issue of *Neuron*]; Anderson et al., 2001). Additionally, a distinct rostral migration of neurons from the striatum is the source of olfactory bulb GABAergic interneurons (Lois et al., 1996; Bulfone et al., 1998; Goldman and Luskin, 1998). Whereas GABAergic neurons are produced from both the LGE and MGE, telencephalic cholinergic neurons appear to derive only from the MGE. There is evidence that cholinergic projection neurons remain in the MGE, whereas cholinergic interneurons tangentially migrate to the LGE/striatum (Marin et al., 2000).

These late migrations of neurons from subpallial regions mean that mutations affecting subpallial development have neuronal deficits in pallial territories. For instance, mutation of the *nkx2.1* gene disrupts MGE specification, completely eliminates telencephalic cholinergic neurons, and greatly reduces the number of cells following the lateral migration pathway to the LGE and cortex (Sussel et al., 1999; Marin et al., 2000; Anderson et al., 2001). Furthermore, mice lacking *dlx1* and *dlx2* (expressed in both the MGE and LGE) have a severe reduction in cortical interneurons derived from both the lateral and rostral migrations (Anderson et al., 1997a, 1997b; Bulfone et al., 1998). In contrast, development of glutamatergic projection neurons in the olfactory bulb and neocortex depends upon local expression of a distinct set of genes, including the T box transcription factor Tbr1, within the pallium (Bulfone et al., 1998; R. Hevner and J. L. R. R., unpublished data). Thus, surprisingly, cortical projection neurons and interneurons have distinct origins and developmental programs.

In addition to defects in early regional patterning of the telencephalon, *pax6* mutant mice also exhibit altered patterns of neuronal migration with many more cells moving from subpallial to pallial regions (Chapouton et al., 1999). However, it is difficult to know whether this phenotype reflects a role for Pax6 in mediating migration, in the establishment of the pallial/subpallial boundary or in the assignment of regional neuronal fates (Stoykova et al., 1996, 2000; Torresson et al., 2000; Yun et al., 2001).

These results suggest that one outcome of early DV patterning of the telencephalon is the specification of domains that produce neurons that synthesize different classes of neurotransmitter. According to this formulation, the cortex produces glutamatergic neurons, the LGE produces GABAergic neurons and the MGE produces both GABAergic and cholinergic neurons (Figure 2C). Each domain produces projection neurons that follow radial migrations and maintain positional information essential for the generation of topographic connectivity maps. Additionally, the LGE and MGE produce interneurons that follow at least two tangential migrations to populate adjacent territories with GABAergic and cholinergic cells. Indeed, the presence of glutamatergic neurons in the basal telencephalon raises the possibility that these cells might also be derived from a distant progenitor zone.

In summary, despite its incredible complexity, considerable progress is being made in elucidating the genetic pathways that underpin the development of functional and morphological subdivisions of the vertebrate telencephalon. The identification of genes that regulate telencephalic induction and patterning opens the door to important new areas of investigation. These include understanding how the various genes interact to regulate evolutionarily conserved aspects of telencephalic development and how divergent characteristics of telencephalic anatomy and function have evolved. To fully understand these processes, progress now needs to be made in identifying the genetic targets of the signaling proteins and transcription factors that regulate regional

and neuronal specification, differentiation, migration, axon pathfinding, and target choice.

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